Fig. 1

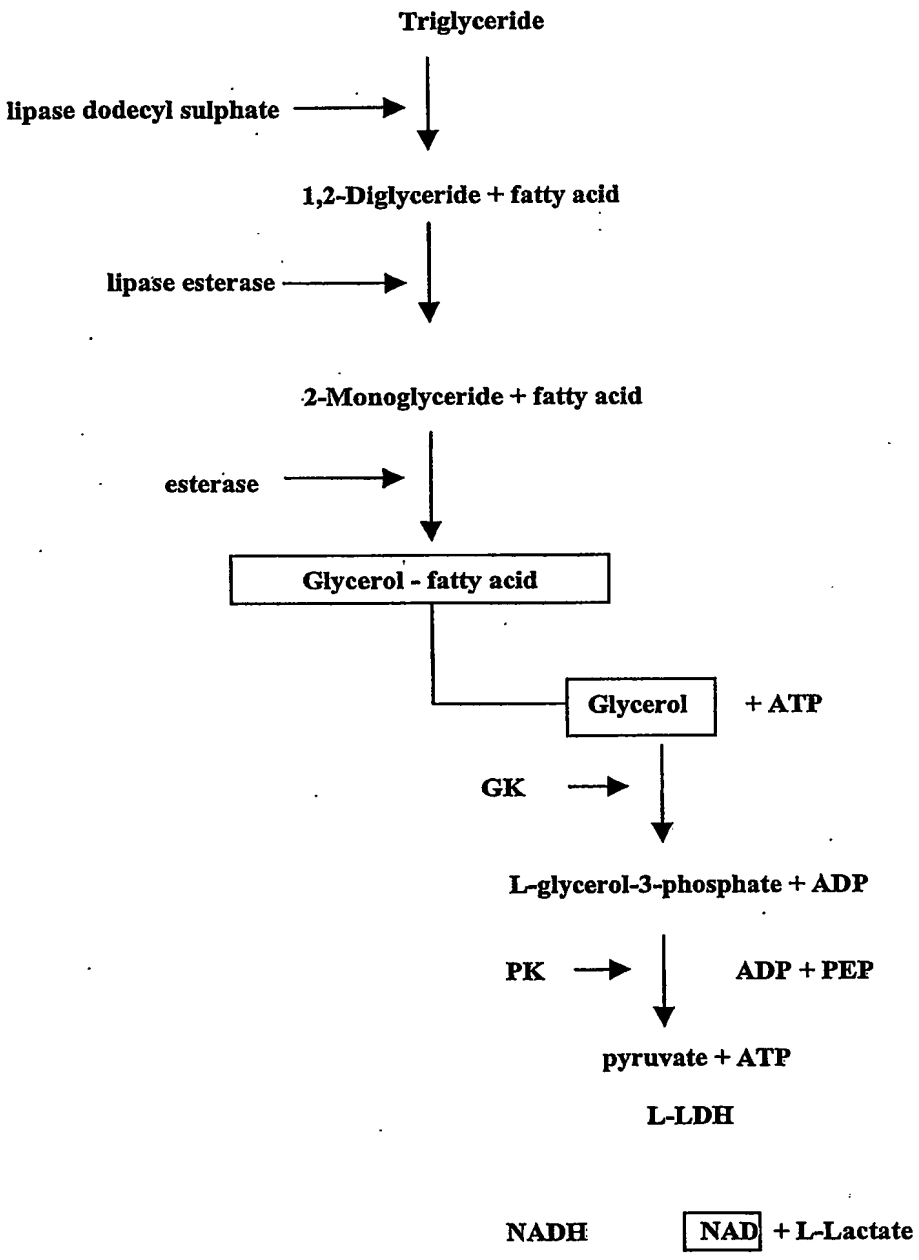

Triglyceride
lipase dodecyl sulphate
1,2-Diglyceride + fatty acid
lipase esterase
2-Monoglyceride + fatty acid
esterase
Glycerol - fatty acid
Glycerol
+ ATP
GK
L-glycerol-3-phosphate + ADP
PK
ADP + PEP
pyruvate + ATP
L-LDH
NADH
NAD
+ L-Lactate

Fig. 2

Novel human membrane glycoprotein

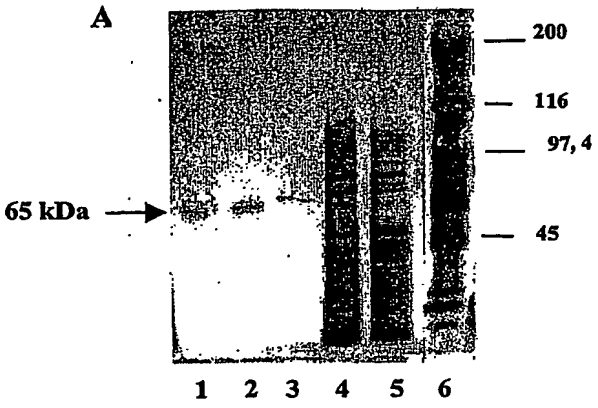


Fig. 3

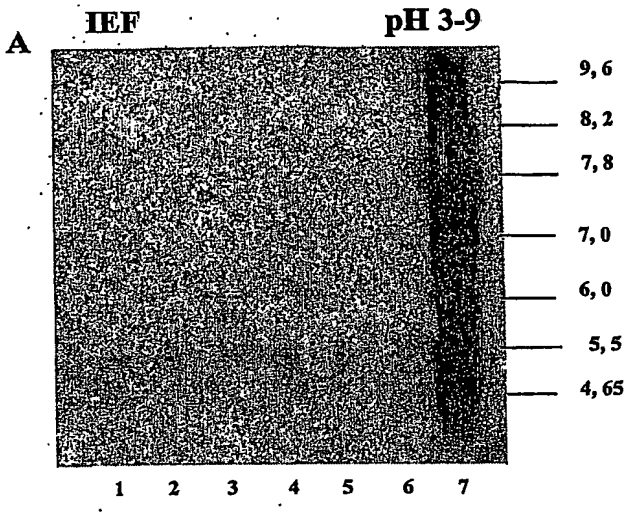


Fig. 4

Peptide A: D-L-V-P-L-E-D-K-V-T-I-L-G-M-T-A

Peptide B: K-L-A-L-S-A-D-D-P-G-F-H-N-F-S-H-Q-R-Q-T

Peptide C: D-Q-Q-T-T-S-H-S-S

Peptide D: V-L-E-I-M-L-P

Peptide E: F-Q-D-E-S-E-A-N-K

Peptide F: M-K-Y-V-N-F-K-F-Y-F

Peptide G: N-L-D-F-M-T-W-G-V-T-K-V-T-Y-I-G-Q-P-T-G-G

Peptide H: L-L-M-D-N-N-E-A-V-H

Peptide I: F-D-Q-A-W-A-D-T-A-H-T-W

Peptide J: K-L-D-D-I-Q-K-D-M-Y-S-Q-Q-D-T

Peptide K: G-V-W-I-M-K-N-Q-I-T

Fig. 5

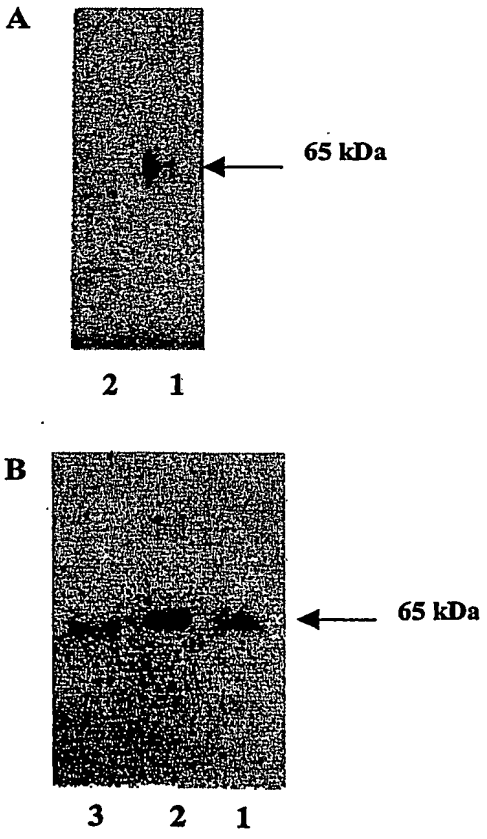


Fig. 6

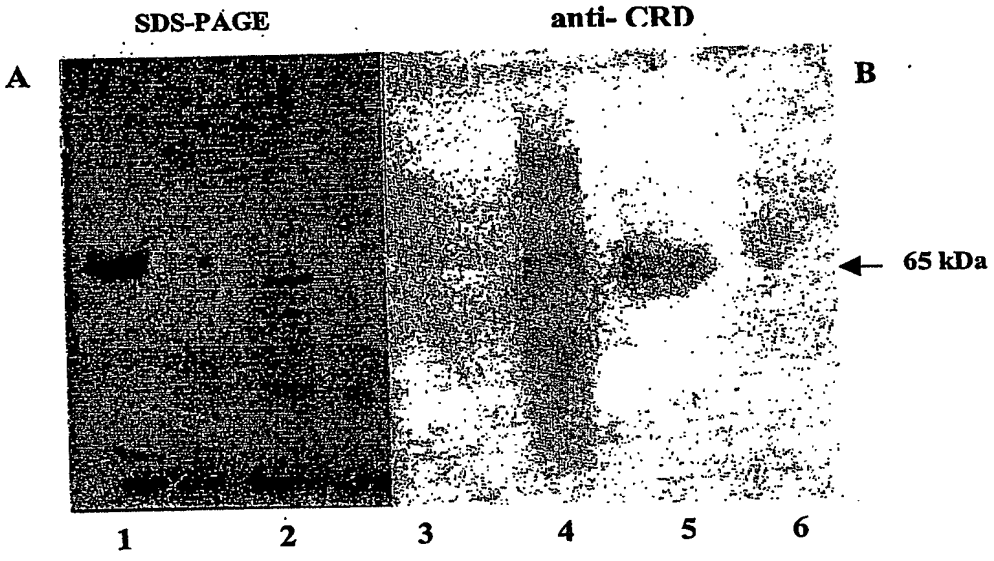


Fig. 7

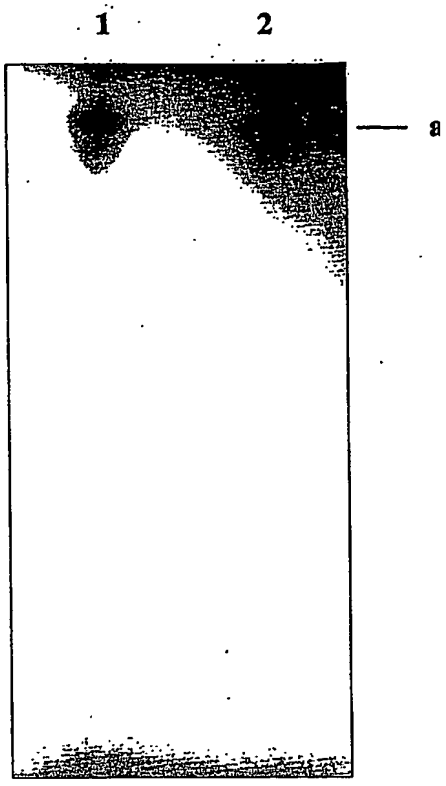
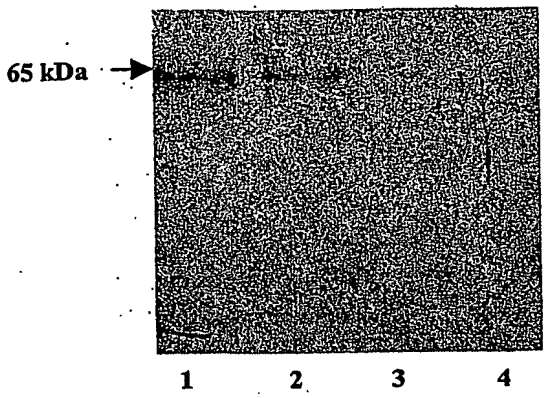


Fig. 8



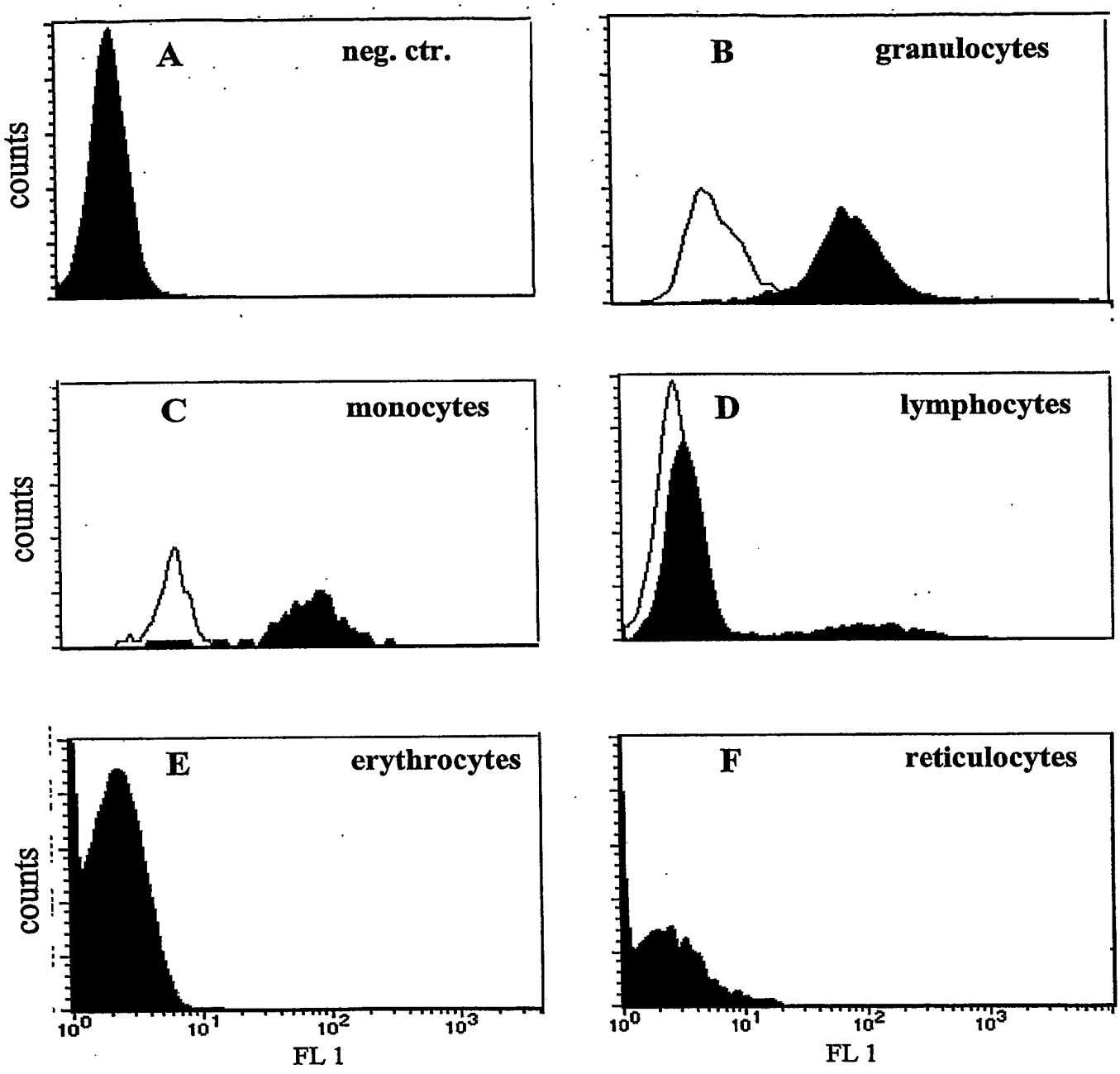

A
neg. ctr.
B
granulocytes
C
monocytes
D
lymphocytes
E
erythrocytes
F
reticulocytes
counts
counts
counts
10^0
10^1
10^2
10^3
FL 1
10^0
10^1
10^2
10^3
FL 1

Fig. 9

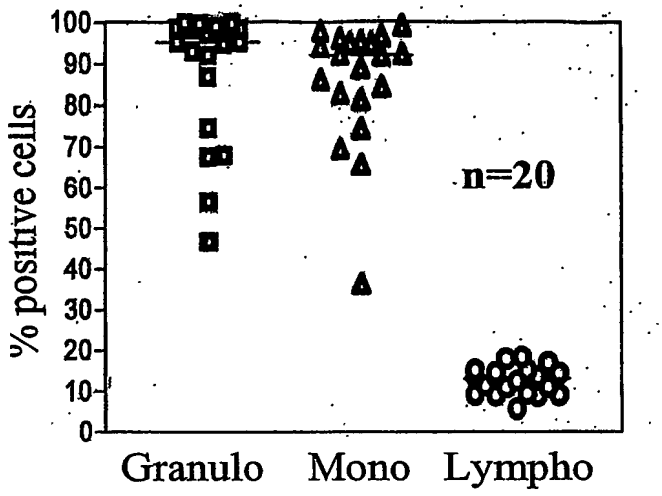
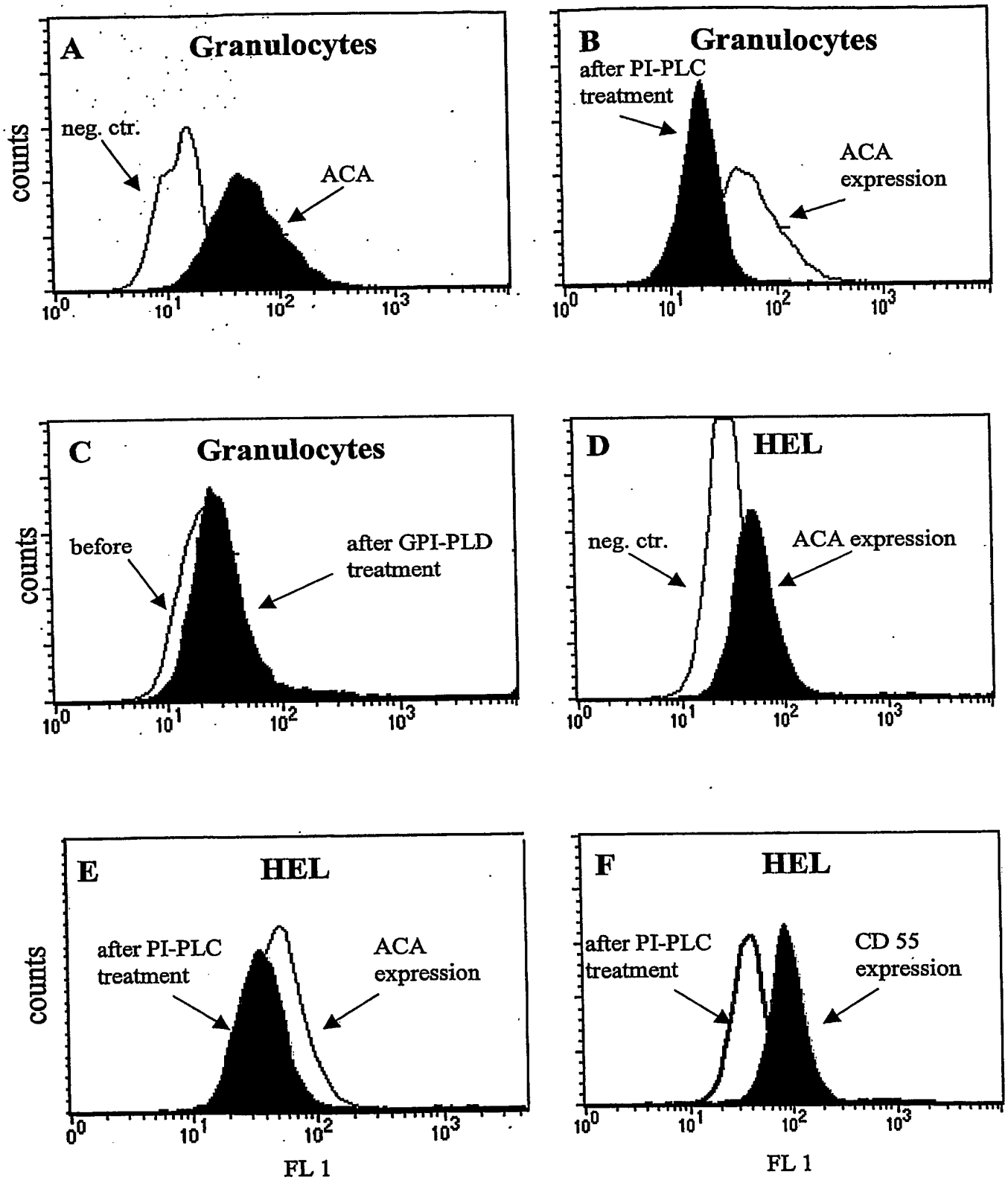

% positive cells
100
90
80
70
60
50
40
30
20
10
0
n=20
Granulo
Mono
Lympho

Fig. 10

Fig. 11


A
Granulocytes
neg. ctr.
ACA
counts
B
Granulocytes
after PI-PLC
treatment
ACA
expression
C
Granulocytes
before
after GPI-PLD
treatment
counts
D
HEL
neg. ctr.
ACA expression
E
HEL
after PI-PLC
treatment
ACA
expression
counts
FL 1
F
HEL
after PI-PLC
treatment
CD 55
expression
FL 1

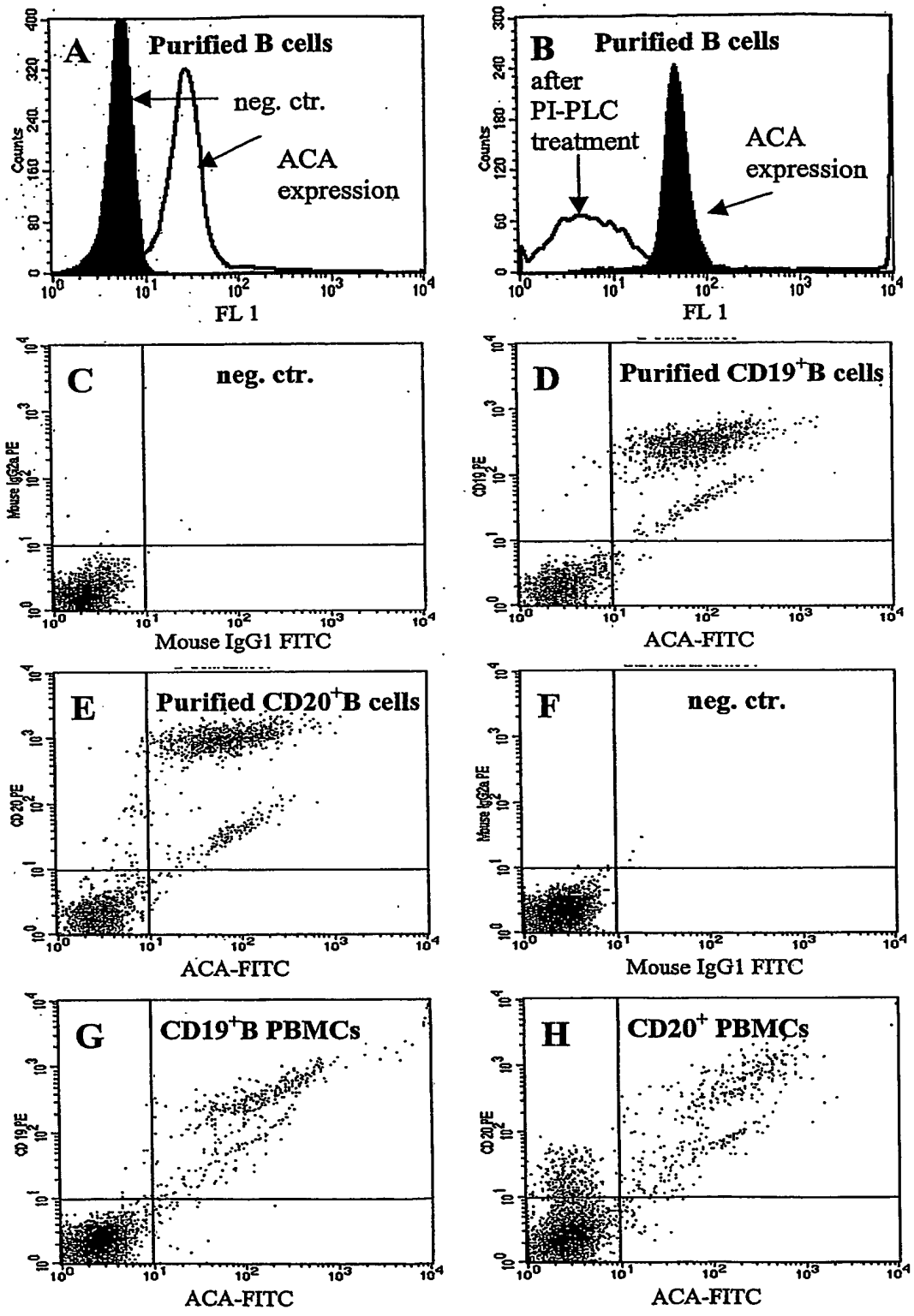

A
Purified B cells
neg. ctr.
ACA expression
Counts
FL 1
B
Purified B cells
after PI-PLC treatment
ACA expression
Counts
FL 1
C
neg. ctr.
Mouse IgG2a PE
Mouse IgG1 FITC
D
Purified CD19+B cells
CD19 PE
ACA-FITC
E
Purified CD20+B cells
CD20 PE
ACA-FITC
F
neg. ctr.
Mouse IgG2a PE
Mouse IgG1 FITC
G
CD19+B PBMCs
CD19 PE
ACA-FITC
H
CD20+ PBMCs
CD20 PE
ACA-FITC

Fig. 12

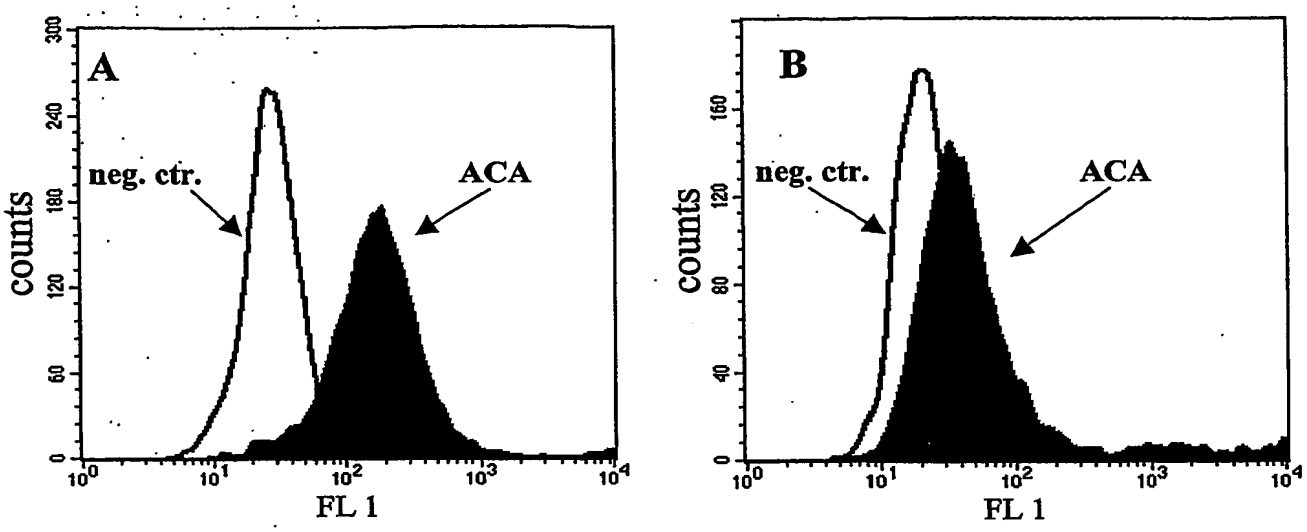


Fig. 13

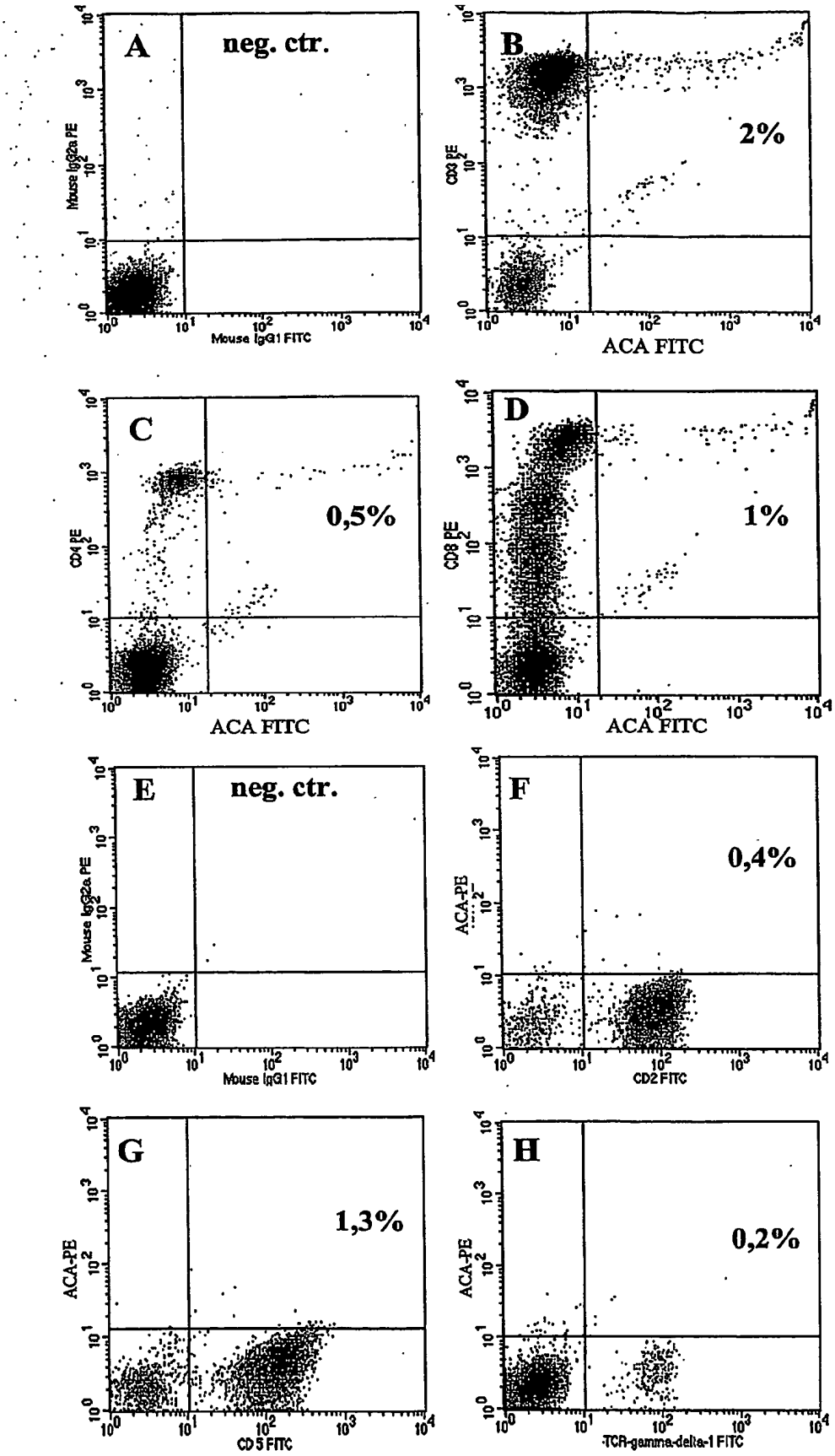

A
neg. ctr.
Mouse IgG2a PE
Mouse IgG1 FITC
B
2%
CD3 PE
ACA FITC
C
0,5%
CD4 PE
ACA FITC
D
1%
CD8 PE
ACA FITC
E
neg. ctr.
Mouse IgG2a PE
Mouse IgG1 FITC
F
0,4%
ACA-PE
CD2 FITC
G
1,3%
ACA-PE
CD 5 FITC
H
0,2%
ACA-PE
TCR-gamma-delta-1 FITC

Fig. 14

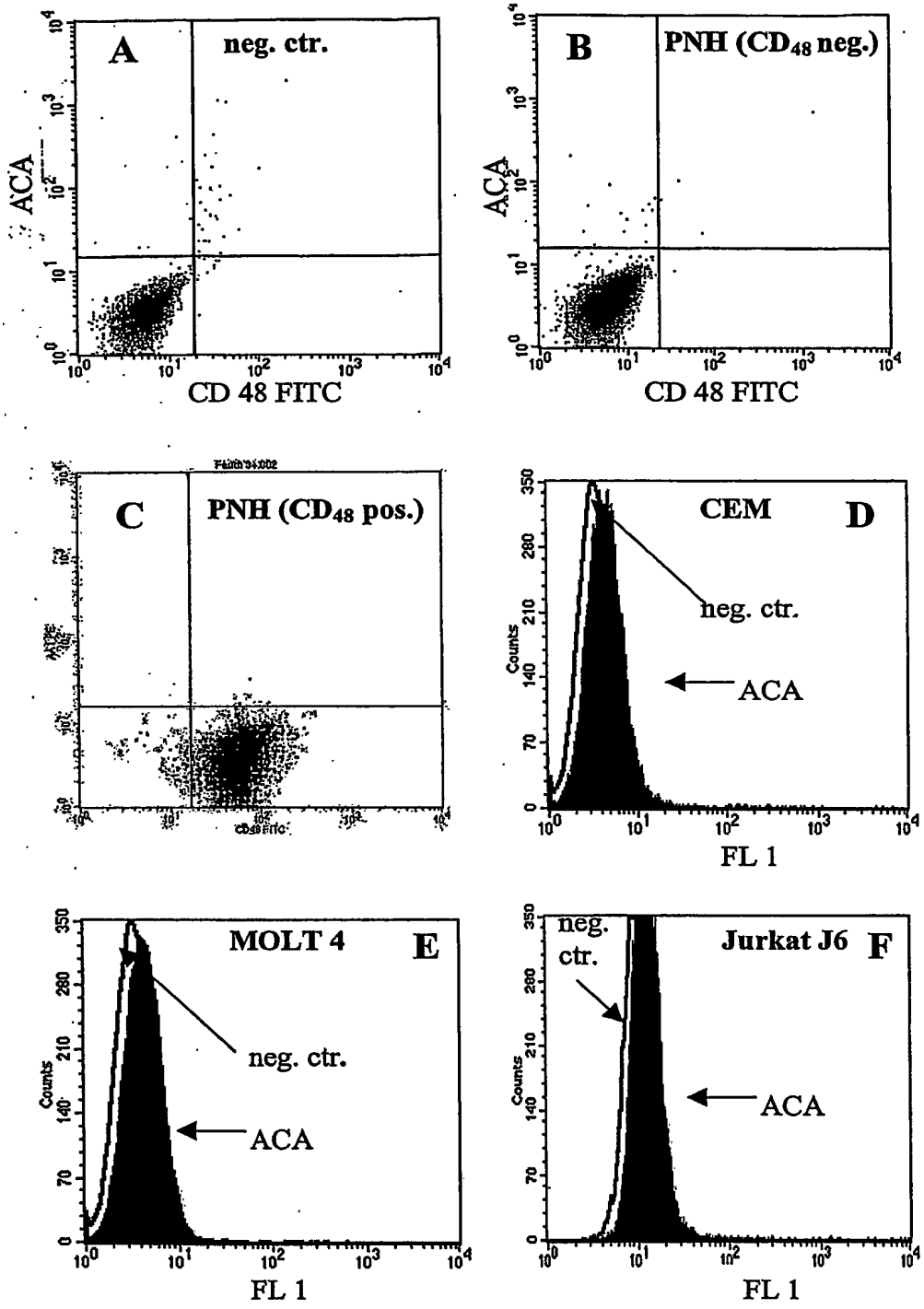

A
neg. ctr.
ACA
CD 48 FITC
B
PNH (CD48 neg.)
ACA
CD 48 FITC
C
PNH (CD48 pos.)
D
CEM
neg. ctr.
ACA
Counts
FL 1
MOLT 4
E
neg. ctr.
ACA
Counts
FL 1
neg. ctr.
Jurkat J6
F
ACA
Counts
FL 1

Fig. 15

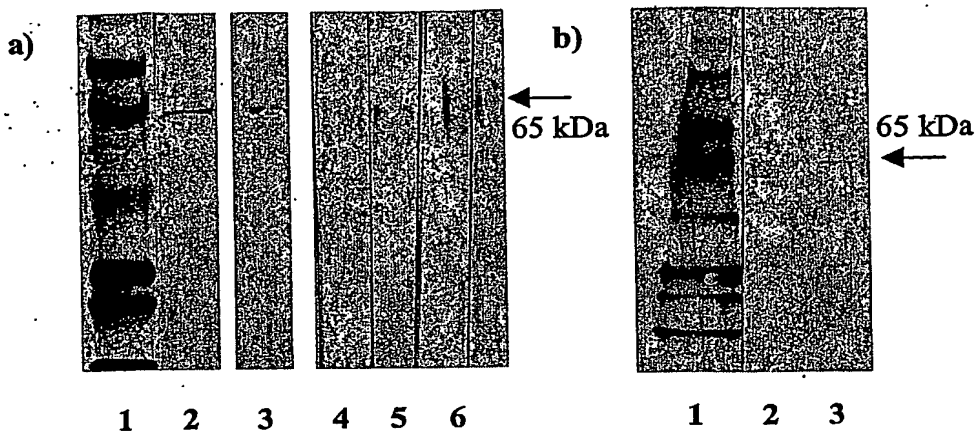
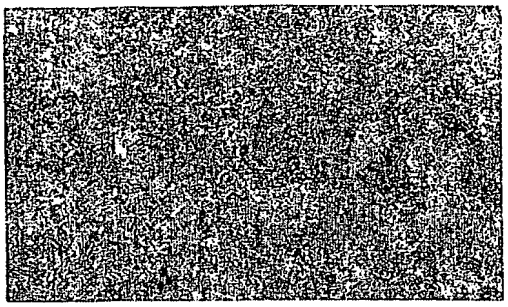


Fig. 16

Fig. 17

a)



b)

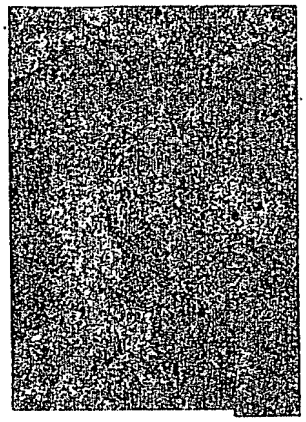


Fig. 17: Frozen sections of normal human skin stained with anti-ACA antibody.

Fig. 18

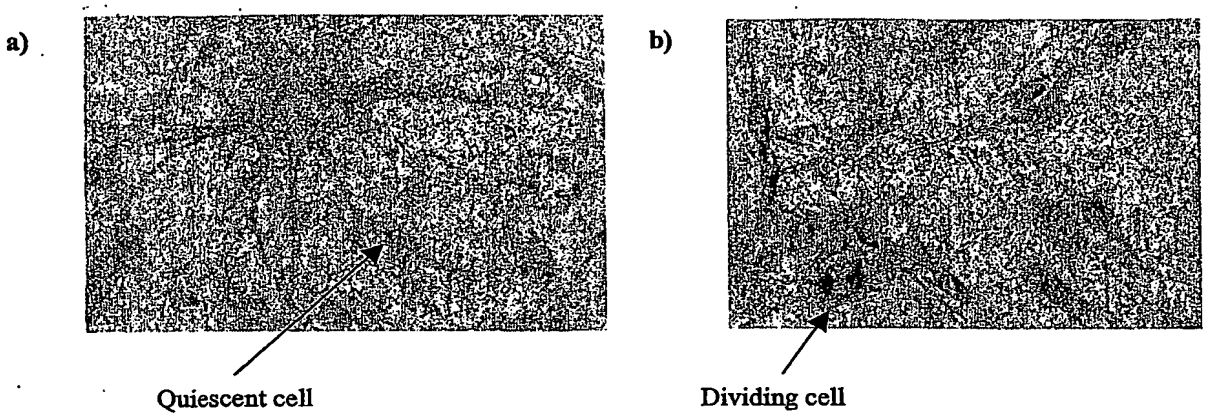
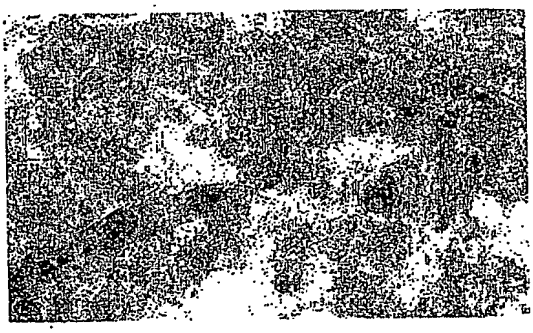


Fig. 18: Normal human cultured epidermal melanocytes stained with anti–ACA antibody.

Fig. 19 a/b

a)



b)

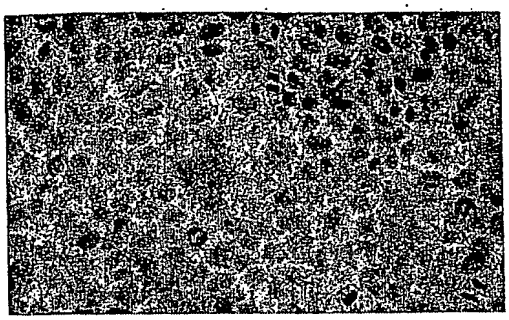


Fig. 19: Normal human cultured epidermal keratinocytes (a) and keratinocytes cell line HaCaT (b) stained with anti –ACA antibody.

Fig. 19c

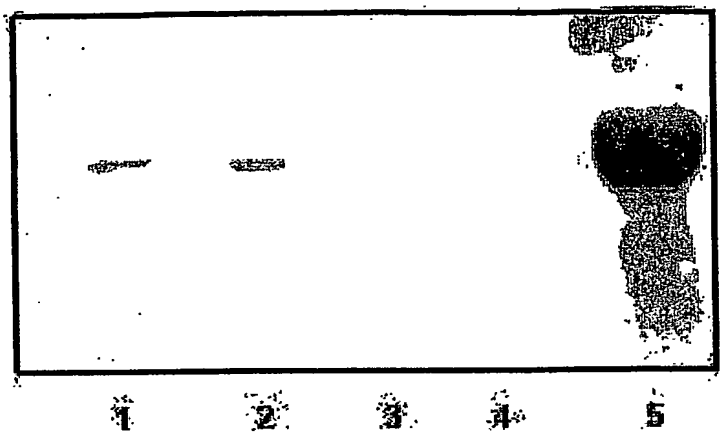


Fig. 19c: Immuno-blot analysis with anti-ACA antibody of cell- free extracts of melanocytes (lanes 1 and 2), keratinocytes (lanes 3 and 4), molecular weight marker (lane 5).

Fig. 20

Fig. 20: Congenital Naevus

Fig. 21

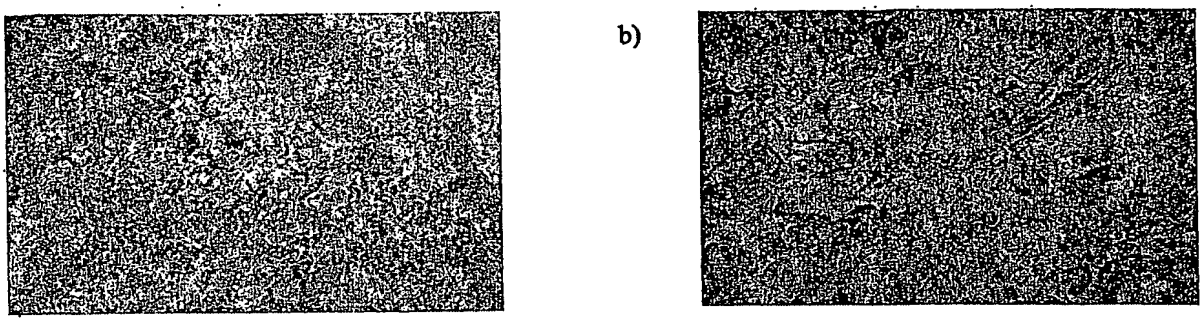


Fig. 21:Frozen sections of human melanoma stained with anti-ACA antibody

Fig. 22

a) b)

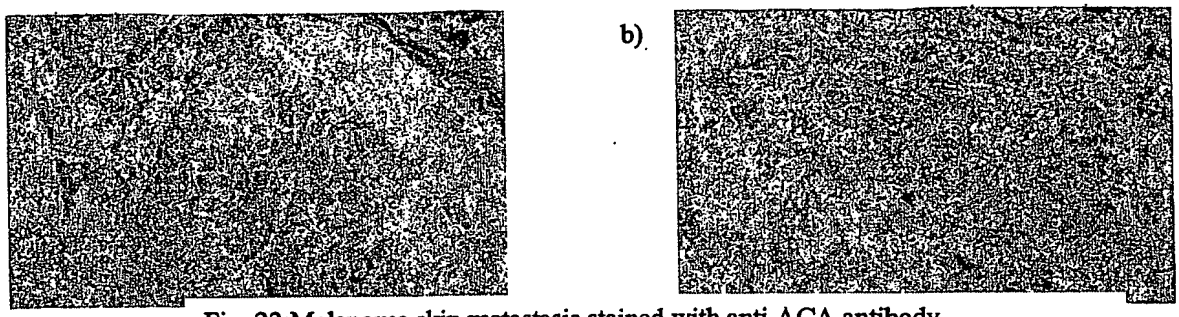


Fig. 22:Melanoma skin metastasis stained with anti-ACA antibody.

Fig. 23

a) b)

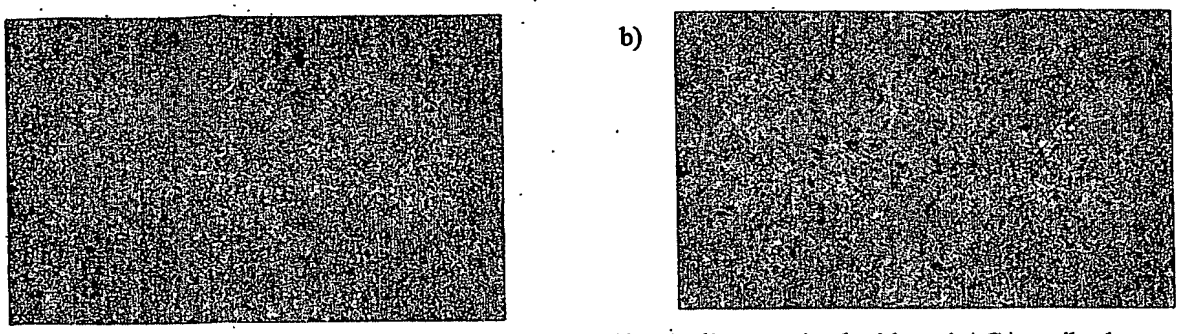


Fig. 23: Frozen sections of human a) basalioma, b) spinalioma stained with anti-ACA antibody.

Fig. 24

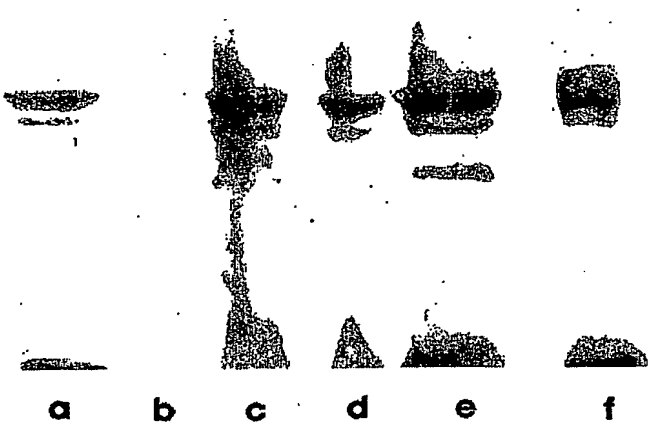


Fig.24: Immunoblot analysis of normal skin and melanoma tumour tissues with anti-ACA antibody: Homogenized normal skin (a), non-immune IgG (neg. ctr.) (b), homogenized melanoma tumour tissues obtained from different patients (c-f).

Fig. 25

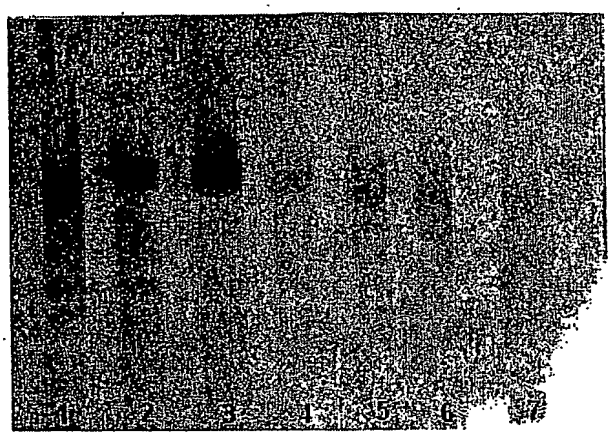
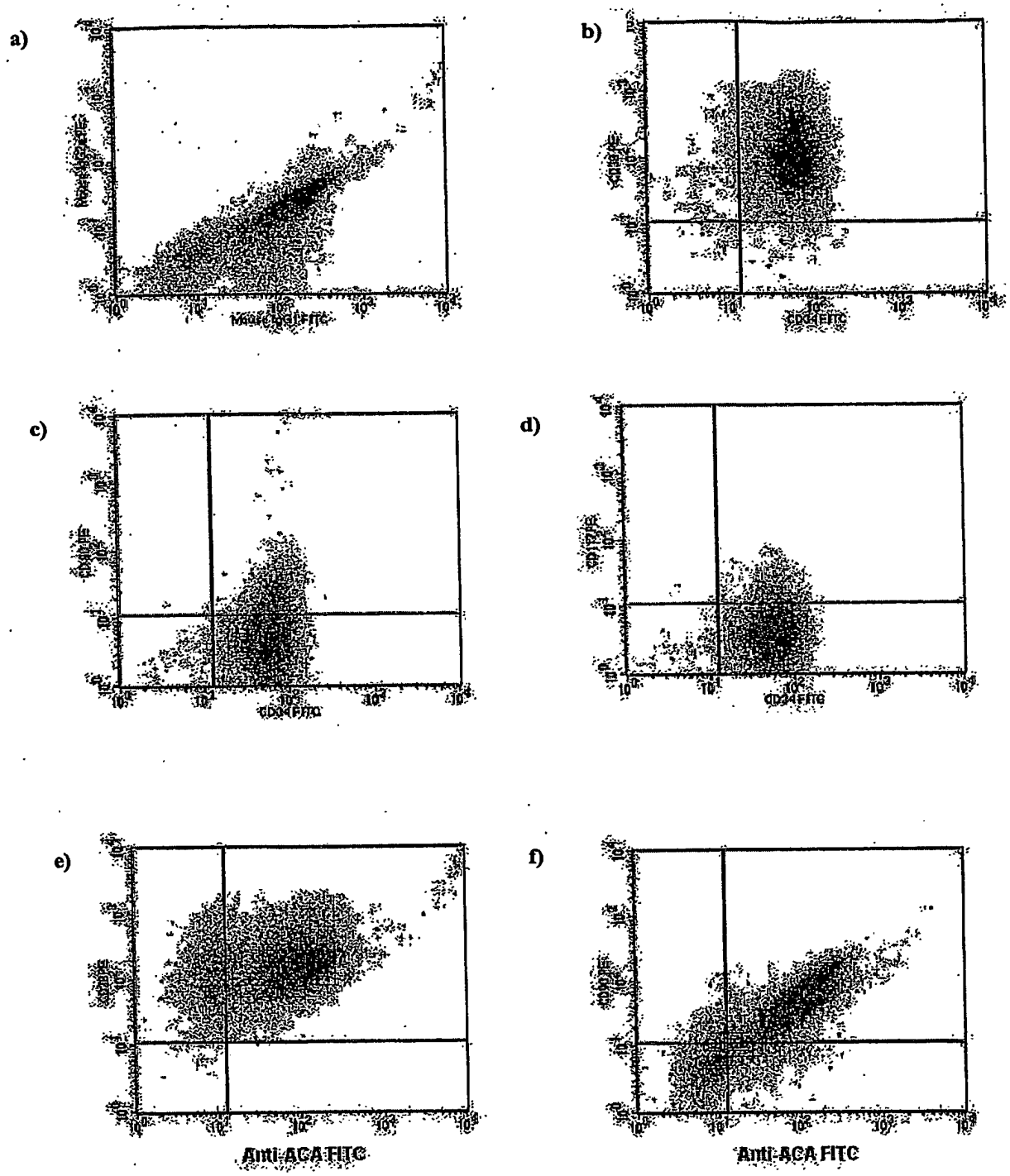


Fig. 25: Immunoblot analysis of homogenized tumour tissues with anti-ACA antibody: Renal (1), Lung (2), Breast (3), Colon (4), Gastric cancer (5), Melanoma (6) and Myeloma (7).

Fig. 26



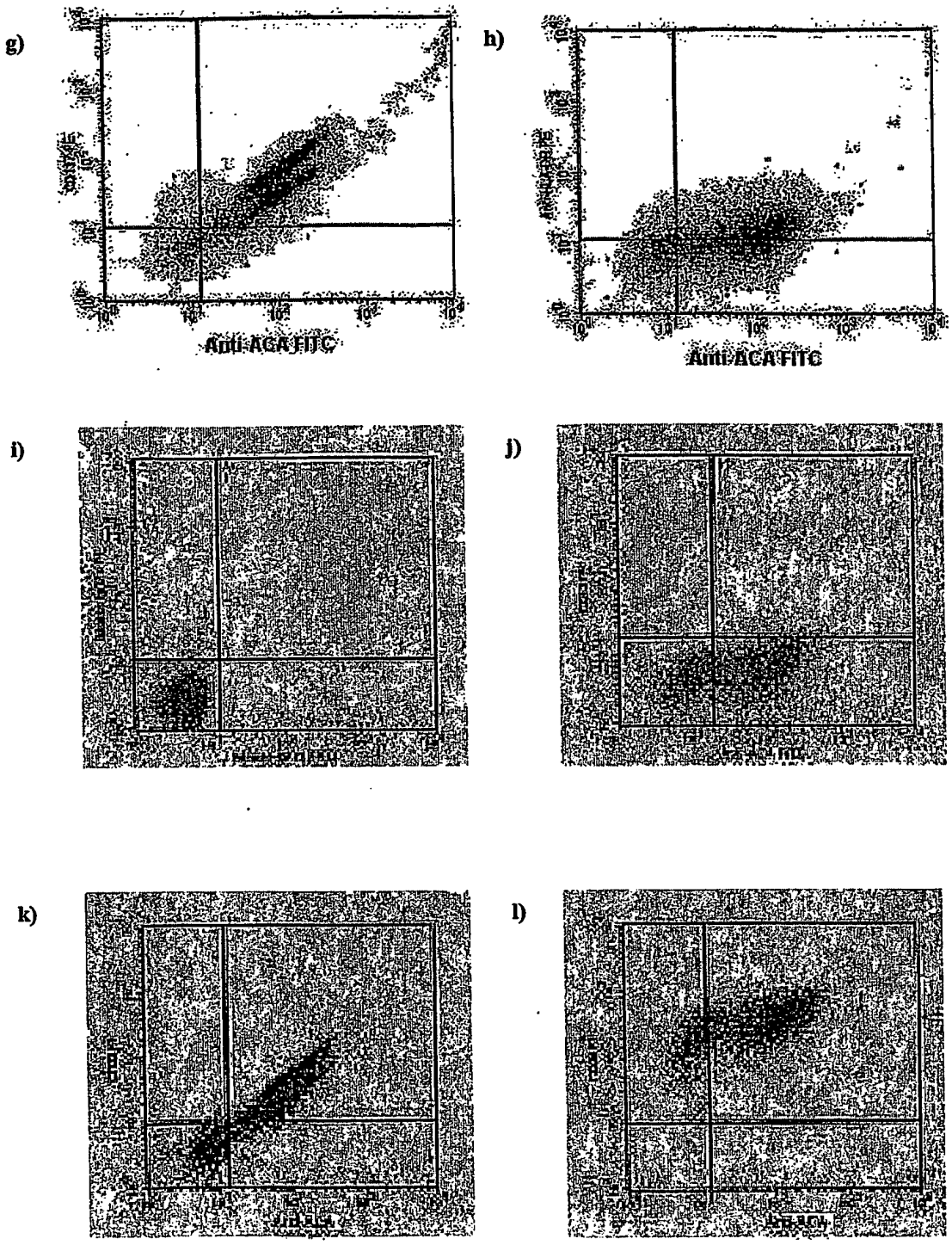


Fig.26: ACA is expressed on stem cell: non-immune IgG (a), CD34/CD38 (b), CD34/CD90 (c), CD34/CD117 (d), anti-ACA/CD38 (e), anti-ACA/CD90 (f), anti-ACA/CD117 (g), anti-ACA/HLA-DR (h), non-immune IgG (i), anti-ACA/CD13 (j), anti-ACA/CD33 (k), anti-ACA/CD34 (l).

Fig. 27

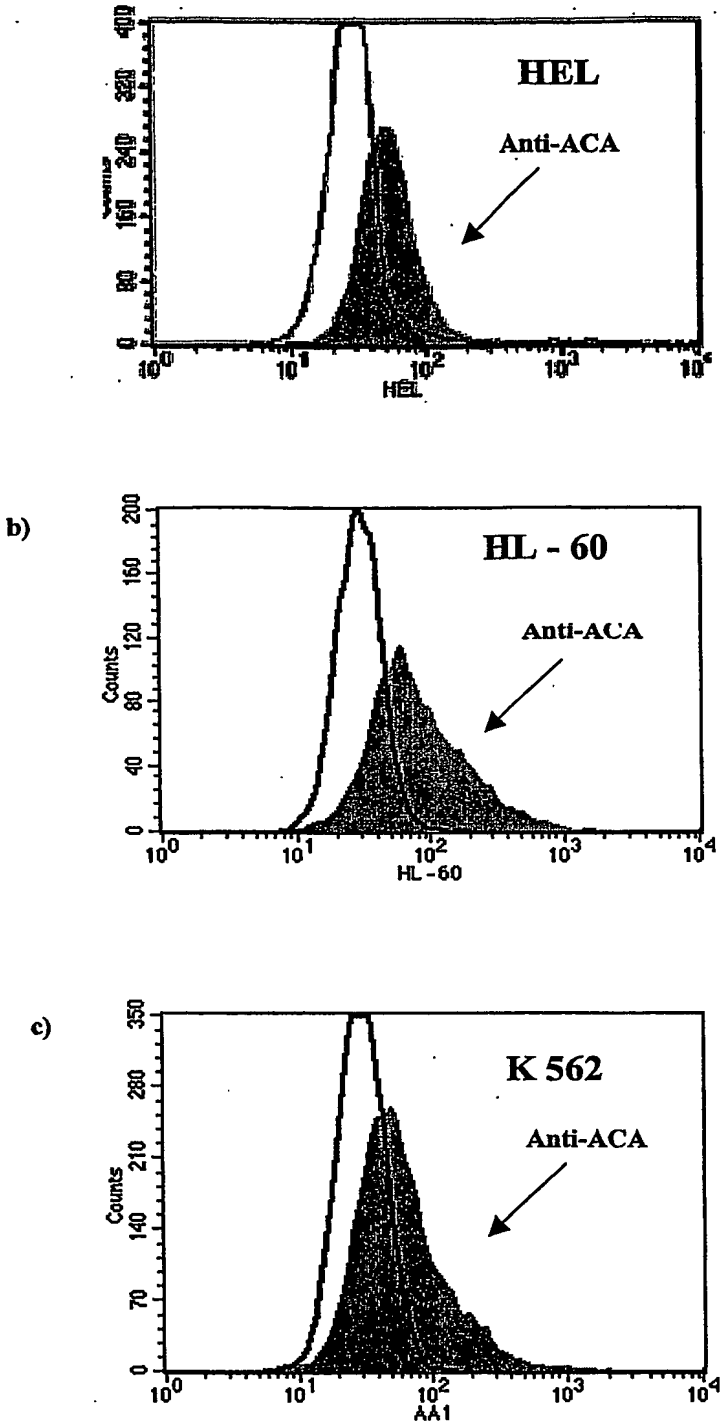


Fig. 27 Flow cytometry analysis of ACA – expression on human leukemia cell lines: (a) erytroleukemia (HEL), (b) promyelocytic erythroleukemia (HL- 60), (c) chronic myelogenous leukemia (K562).

Fig. 28

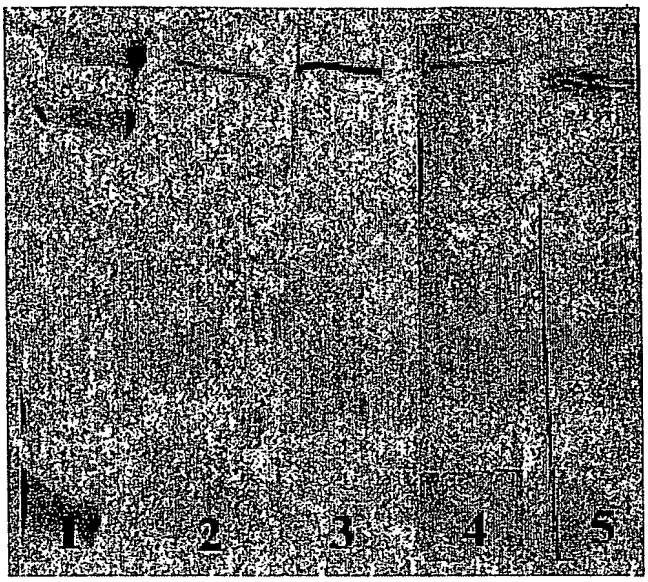
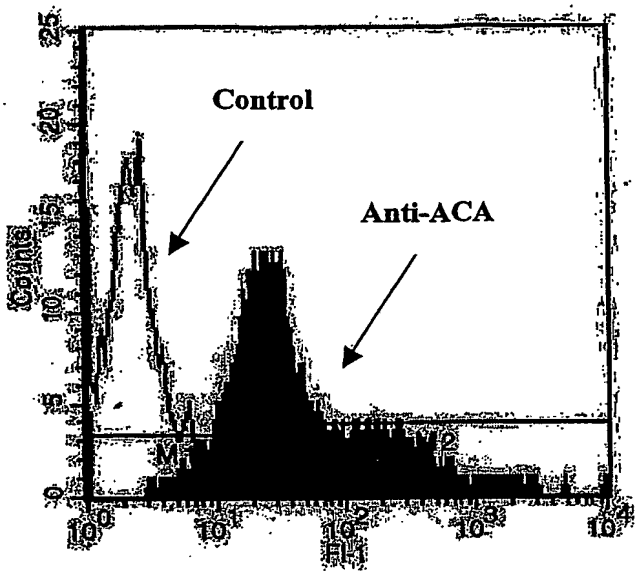


Fig. 28:Immunoblot analysis with anti-ACA antibodies of cell-free extracts of human leukemia cell lines: molecular weight marker (1), human chronic myelogenous leukaemia (k-562) (2), human promyelocytic erythroleukemia (HL-60) (3), human erythroleukemia (HEL) (4), human histiocytic lymphoma (U-937) (5).

Fig. 29 a/b

a)



b)

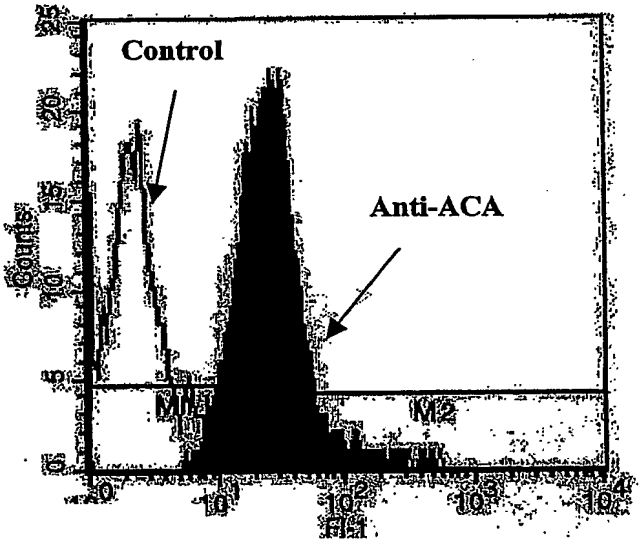


Fig. 29a/b: Peripheral blood cells of PNH patients were incubated with mouse monoclonal antibodies to ACA plus anti-mouse-IgG FITC and analysed by FACS. After lysis of erythrocytes the granulocytes were gated by FSC/SSC.

Fig. 29 c

c)

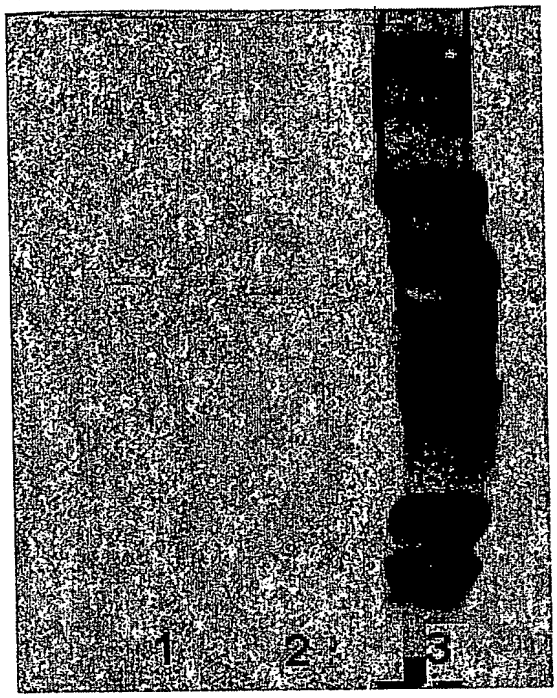


Fig. 29c: Immunoblot analysis with anti-ACA antibody of granulocytes membrane protein fraction obtained from healthy donor (lane 1), versus PNH patient (lane2).

Fig. 29 d

d)

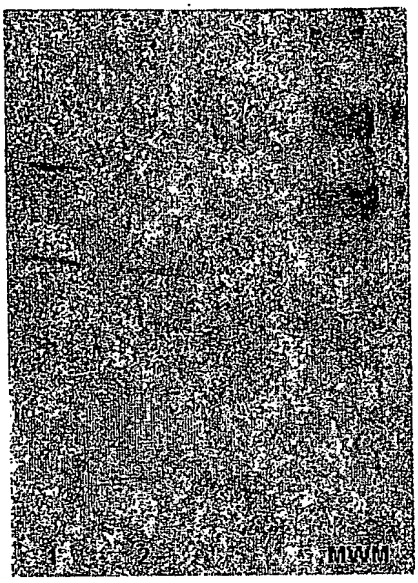


Fig. 29d: Immunoblot analysis with anti-ACA antibody of PNH granulocytes membrane fraction before (lane 1), and after treatment with phospholipase C (lane 2), molecular weight marker (line 3).

Fig. 29 e/f

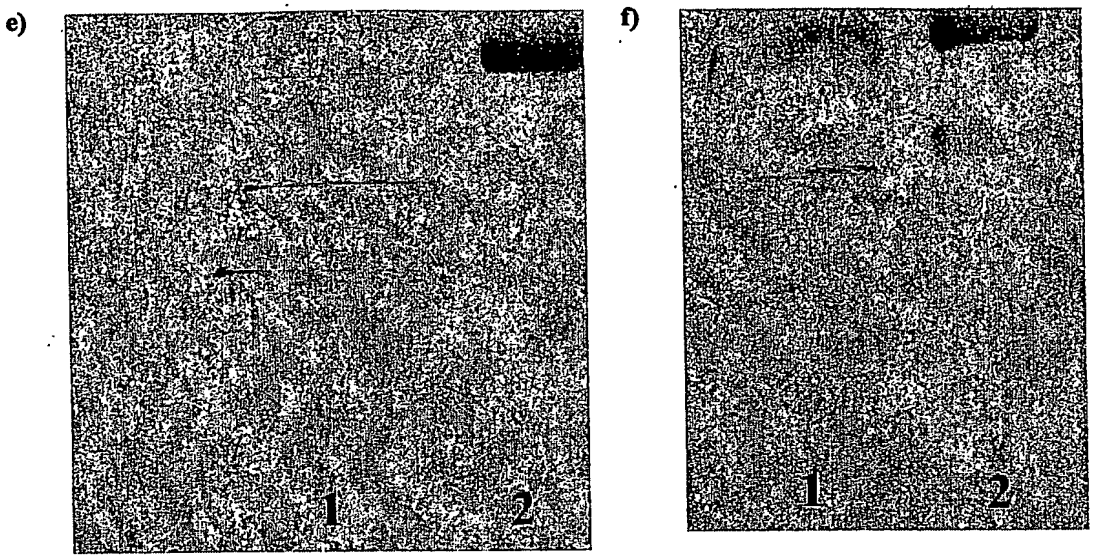


Fig. 29 e,f: Immunoblot analysis of soluble form of ACA in supernatant of granulocytes derived from healthy donor (d) and PNH patient (e) after the action of phospholipase C.

Fig. 30

2 1

Fig.30 Purification and isolation of ACA proteins. Electrophoresis of purified ACA proteins was performed on 4-15% SDS-PAGE. *Lane 1*, purified main form of ACA protein, molecular mass 65 kD; *lane 2*, purified ACA protein molecular mass 68 kD.

Fig. 31

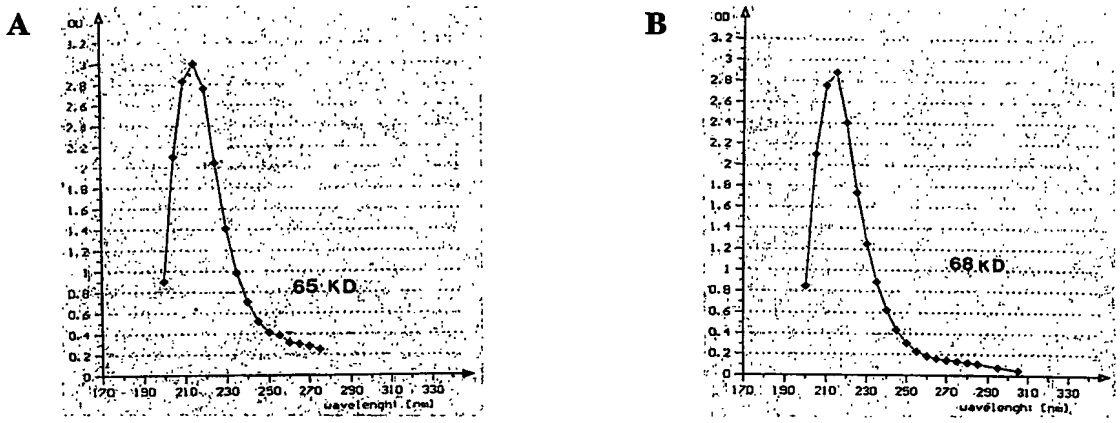


Fig. 31 **UV spectrum of isolated ACA proteins.** UV spectrum of purified two forms of erythrocyte ACA protein was read in Beckman spectrophotometer. A, UV spectrum of purified 65 kD ACA protein; B, UV spectrum of 68 kD ACA protein.

Fig. 32

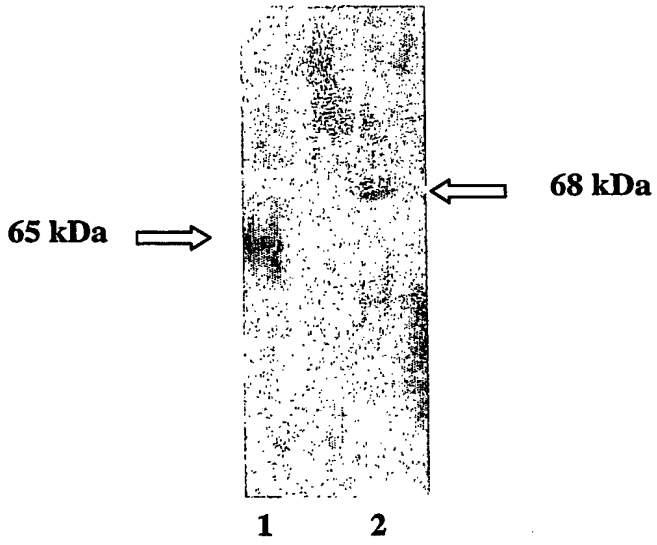


Fig. 32 Western blotting with anti-ACA polyclonal antibody
Proteins were subjected to SDS-PAGE under reducing condition transferred to nitrocellulose, and revealed with mouse antibody to ACA. *Lane1,* purified 65 kD erythrocyte ACA protein; *lane 2*, purified 68 kD erythrocyte ACA protein

Fig. 33

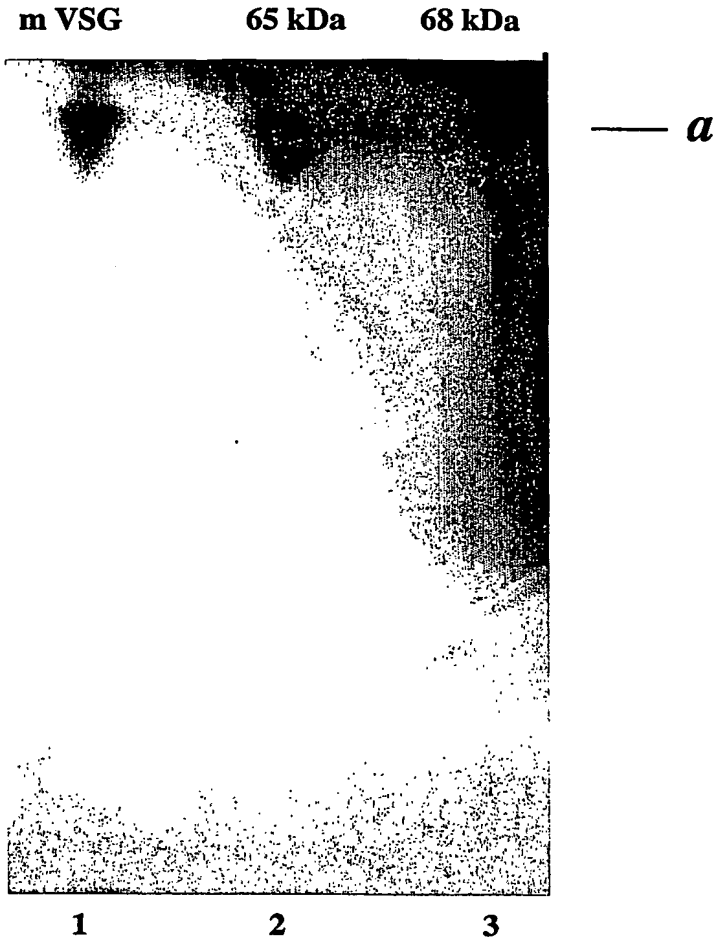


Fig. 33 **Silica TLC analysis of fragments generated by hydrolysis of anchors.** Samples of [^{125}I] TID-labeled, purified proteins were hydrolyzed with GPI-PLC. The lipid products of this reaction were further hydrolyzed with highly specific lipases. Radiolabeled fragments were extracted and analyzed by TLC. Myristic acid was used as standard. *Lane 1*, commercially available mVSG used as control was labeled, digested with GPI-PLC and further hydrolyzed as described for ACA; *lane 2*, 65 kD ACA protein digested with GPI-PLC and further hydrolyzed; *lane 3*, 68 kD molecular mass form of ACA digested and further hydrolyzed as already described.

Fig. 34

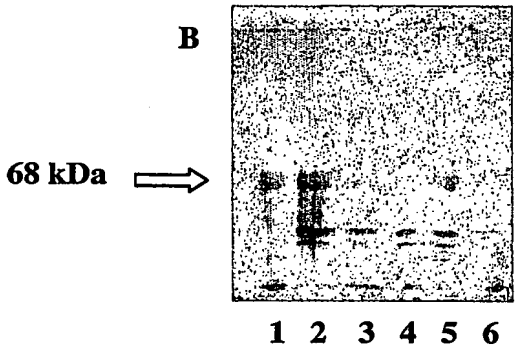


Fig. 34B **SDS-PAGE analysis of 68 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods.** *Lane 1* purified 68 kD ACA after incubation with PNGase F for O min; *lane 2*, purified 68 kD ACA protein incubated with PNGase F for 10 min; *lane 3*, 30 min; *lane 4*, 90 min; *lane 5*, for 150 min; *lane 6,* overnight.

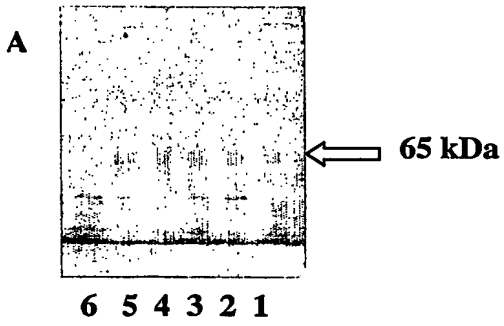


Fig. 34A **SDS-PAGE analysis of 65 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods.** *Lane 1* purified 65 kD ACA after incubation with PNGase F for O min, *lane 2*, purified 65 kD ACA protein incubated with PNGase F for 10 min, *lane 3*, 30 min, *lane 4,* 90mins, *lane 5*, for 150 min, *lane 6*, overnight.

Fig. 35

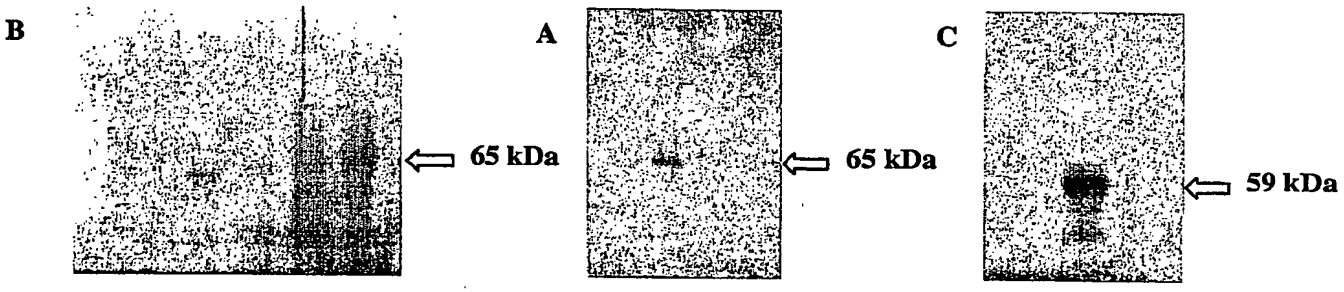


Fig. 35 **Enzymatic and chemical deglycosylation of purified 65 kD erythrocyte ACA.** Electrophoresis of purified 65 kD ACA protein and the products of its enzymatic and chemical deglycosilations was performed on 4-15% SDS-PAGE. *A, lane 1*, purified 65 kD ACA. *B, lane 1*, purified 65 kD ACA treated with sialidase, *lane 2*, O-glycosidase, *lane 3*, PNGase F, *lane 4*, subsequent treatment of 65 kD erythrocyte ACA with sialidase O-gycosidase and PNGaseF. *B, lane 1* purified 65 kD ACA protein after a chemical deglycosilation using TMSF.